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The human ubiquitin-conjugating enzyme UbcH1 is involved in the repair of UV-damaged, alkylated and cross-linked DNA

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Abstract

The human ubiquitin-conjugating enzyme UbcH1 shows 69% identity to the *Saccharomyces cerevisiae* RAD6/UBC2 which plays a key role in DNA repair. To examine the function of UbcH1 (formerly named E2, M_r 17 000), [(1990) EMBO J. 9, 1431–1435]) we tested its ability to functionally substitute for yeast RAD6/UBC2 in the recovery of cells from various DNA damage. Complementation by expression of the human *UbcH1* cDNA revealed that the UbcH1 carries out the function of *S. cerevisiae* RAD6/UBC2 in the repair of UV-damaged, alkylated and cross-linked DNA.

Key words: DNA repair; Ubiquitin conjugation

1. Introduction

Ubiquitin is present in all eukaryotic cells and is the most conserved protein known so far. It is covalently attached to many cellular proteins and targets them to selective degradation. However, the presence of stable ubiquitin conjugates of histones H2A and H2B in chromatin suggests an alternative, non-proteolytic role for ubiquitination, in which its reversible attachment to other proteins modulates their function by structural changes (recently reviewed in [1–3]).

Ubiquitination of proteins requires activation of ubiquitin by an activating enzyme (E1) and substrate-specific conjugation by ubiquitin-conjugating enzymes (E2) [4]. One of these E2 enzymes is the *Saccharomyces cerevisiae* RAD6/UBC2 [5]. The *RAD6/UBC2* gene plays a key role in DNA repair, induction of mutagenesis, and sporulation. Rad6 mutants are hypersensitive to ultraviolet light, alkylating and cross-linking chemicals [6], are totally deficient in mutagenesis induced by these agents, and fail to sporulate. In cooperation with the yeast ubiquitin ligase (E3), RAD6/UBC2 ubiquitinates proteins depending on their N-terminal amino acids, subjecting them to protein degradation by the N-end rule pathway [7,8]. However, the RAD6/UBC2 protein monoubiquitinates and polyubiquitinates the histones H2A and H2B in vitro in an E3-independent reaction [5], and the highly acidic C-terminal extension of RAD6/UBC2 is required for this reaction [9].

A remarkably conserved human homologue of RAD6/UBC2 was originally isolated by Schneider et al. [14], followed by reports of homologous enzymes in *Schizosaccaromyces pombe* [10], *Drosophila melanogaster* [11], *Arabidopsis thaliana* [12], and rabbit [13]. In human the existence of isoenzymes has been reported which share 95% identity at the amino acid level [15]. Although the human homologues show about 70% identity compared with RAD6/UBC2, they completely lack the above-mentioned C-terminal acidic extension, which may result in functional differences. Interestingly, very recently we identified a human ubiquitin-conjugating enzyme (UbcH2) containing a C-terminal extension enriched in acidic residues which is also able to ubiquitinate histones [16].

In addition to UbcH1's role in repair of UV-damaged DNA (as shown by Koken et al. [15]), we were interested in whether it is also involved in the repair of alkylated and cross-linked DNA. For this purpose we expressed the cDNA encoding the human UbcH1 in rad6 mutants and assayed for repair of DNA lesions induced by UV light, MMS (methylmethanesulfonate) and 8-MOP (8-methoxypsoralen plus UV-A-light).

2. Experimental

2.1. Yeast strains and growth conditions

The haploid *S. cerevisiae* strains used in this study were the RAD6$^+$ strain YWO2 (*MATa, his3-Δ200, leu2-3, leu2-112, lys2-801, trp1-1(am), ura3-52*) and the rad6Δ strain YWO62 (*MATa, his3-Δ200, leu2-3, leu2-112, lys2-801, trp1-1(am), ura3-52, rad6::HIS3*), both kindly provided by W. Seufert (Universität München). The standard synthetic complete and rich media used have been described elsewhere [17].

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2.2. Construction of expression plasmids

The cDNA clone UI8, encoding the human ubiquitin-conjugating enzyme UbcH1 [14], was digested with *Bam*HI and *Pvu*II. Klenow polymerase was used to fill in the *Bam*HI site of the cDNA. After addition of *Not*I linkers and subsequent digestion with *Not*I, the *UbcH1* insert was separated by agarose gel-electrophoresis from linker fragments and vector sequences. Cloning of the extracted *UbcH1* cDNA fragment into the unique *Not*I site of the *S. cerevisiae* expression vector YEp351-ADCI resulted in plasmid YEp351-ADCI-UbcH1, which allows the expression of the human *UbcH1* cDNA in *S. cerevisiae*.

The expression vector YEp351-ADCI was constructed by cloning the *Bam*HI fragment of plasmid YpNot [18], which contains the *S. cerevisiae* ADCI promoter and terminator separated by a *Not*I site, into the *Bam*HI site of the 2μ-based YEp351 [19].

2.3. Cloning, sequencing and transformation

All plasmids were propagated in the *Escherichia coli* strain SURE (Stratagene) and isolated as described [20]. Cloning and sequencing methods were according to standard protocols [21]. Yeast strains were transformed by a modified lithium acetate protocol [22], and transformants were analysed as described [23].

2.4. Survival assays with S. cerevisiae

Yeast cells were grown in liquid synthetic complete media lacking leucine to mid exponential phase. To assay UV sensitivity, cells were plated on appropriate agar plates, incubated at 30°C for 4 h and irradiated with UV light at a dose rate of 0.5 $J \cdot m^{-2} \cdot s^{-1}$. The plates were incubated in the dark to avoid photoreactivation and surviving colonies were counted after 5–6 days. To measure the capacity to repair alkylated DNA, cells were plated on MMS-containing plates, which were used immediately after preparation. Surviving colonies were counted after 6 days.

To assay viability after treatment with the cross-linking agent 8-MOP, cells were washed three times with phosphate-buffered saline (PBS) and incubated for 30 min at 30°C in PBS containing 5 μM 8-MOP. The 8-MOP-treated cells were placed in a cell culture dish (30 × 20 mm) and exposed to various doses of UV-A light (365 nm) to induce DNA cross-links. Irradiated cells were washed twice with PBS to remove residual 8-MOP and plated on appropriate agar plates. Surviving colonies were counted after 6 days incubation at 30°C.

3. Results and discussion

The highly homologous primary structures of the yeast RAD6/UBC2 and the human UbcH1 as well as complementation experiments suggest similar cellular functions of these ubiquitin-conjugating enzymes [14,15]. We were interested in if the human enzyme UbcH1 has a central role to play in the repair of various DNA lesions, as proposed for RAD6/UBC2. For this purpose, we constructed a yeast/*E. coli* shuttle plasmid (YEp351-ADCI-UbcH1), which allows the constitutive expression of UbcH1 in *S. cerevisiae* (Fig. 1). The expression of the cDNA encoding the human UbcH1 is driven by the yeast ADCI promotor, and the corresponding 3′-untranslated sequences provide proper termination of the transcripts [24]. To test whether the human UbcH1 carries out the RAD6/UBC2 functions, several yeast transformants were made: (i) rad6Δ-UbcH1, a rad6 mutant expressing the human homologue; (ii) rad6Δ-351, the rad6 mutant transformed with plasmid 351-ADCI (expresses no UbcH1); (iii) RAD6$^+$-351, a wild-type yeast carrying 351-ADCI. In an initial experiment we compared the viability of these transformants after UV irradiation. Fig. 2 shows the results of the survival assay. The human UbcH1 significantly increases the resistance of cells to UV radiation. The major UV-induced DNA lesions are cyclobutane pyrimidine dimers and 6-4 photoproducts, which are preferentially repaired by a multistep repair process called nucleotide excision repair (NER) (recently reviewed in [25,26]). In contrast to photoproducts, DNA lesions induced by alkylating agents are repaired by a different pathway, namely base excision repair (BER) [27]. However, rad6 mutants are sensitive to alkylating agents [6] which indicates a connection between RAD6/UBC2 and BER. Involvement of the human UbcH1 in this repair pathway was proven by complementation experiments similar to those involving UV treatment, but with MMS as the mutagenic agent. The viability of rad6 mutants after such treatment was substantially increased by the presence of the human UbcH1 (Fig. 3). At an MMS concentration of 0.025% survival was enhanced about 800-fold. Apparently, the human ubiquitin-conjugating enzyme UbcH1 participates in both of the differing repair processes (NER and BER) necessary to allow cells to recover from UV- as well as MMS-induced DNA damage.

Little is known about the mechanisms involved in the repair of cross-linked DNA induced by photoactivated 8-MOP, but double-strand breakage of DNA seems to be necessary [28]. Rad6 mutants are abnormally sensitive to killing by 8-MOP. Therefore, we investigated if the complementation also extends to cross-link repair mech-

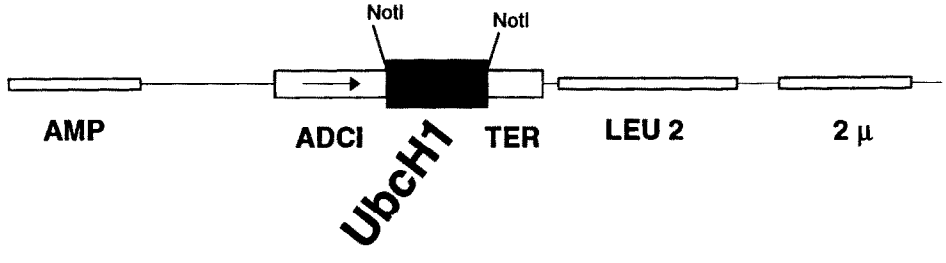

Fig. 1. Schematic representation of the yeast expression construct YEp351-ADCI-UbcH1. AMP, *E. coli* β-lactamase gene conferring resistance to ampicillin; ADCI, alcohol dehydrogenase promoter from *S. cerevisiae*; TER, termination signals of the alcohol dehydrogenase gene from *S. cerevisiae*; UbcH1, cDNA encoding the human UbcH1; LEU2, β-isopropylmalate dehydrogenase from *S. cerevisiae* conferring leucine prototrophy; 2μ, 2μ element.

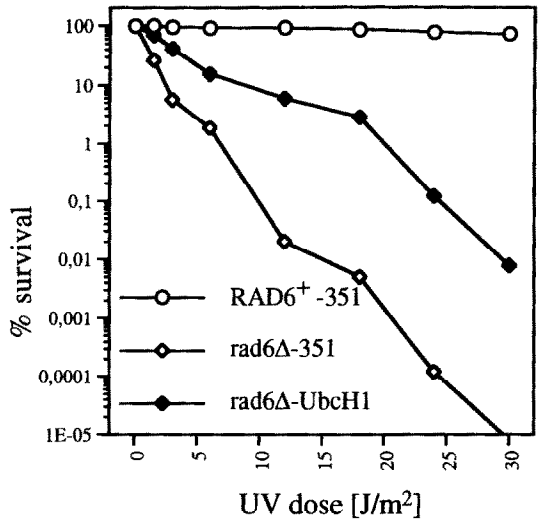

Fig. 2. Survival of yeast transformants after UV irradiation with the indicated dose. Results represent 3 independent experiments. Symbols are indicated in the legend. RAD+-351, wild-type; rad6Δ-351, mutant; rad6Δ-UbcH1, mutant yeast expressing human UbcH1.

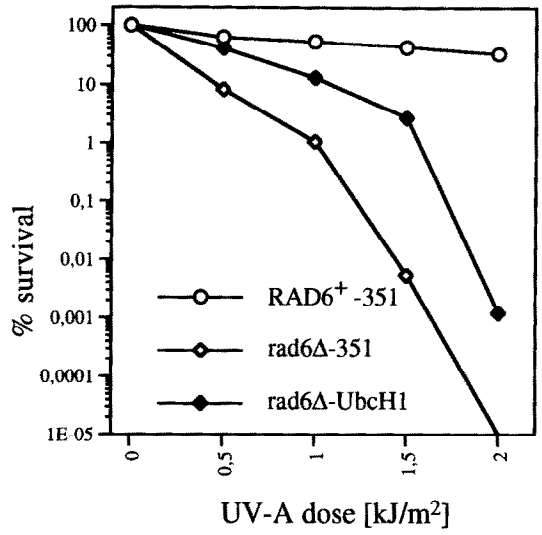

Fig. 4. Survival of yeast transformants treated with 8-methoxypsoralen and the indicated dose UV-A (365 nm). Results represent 3 independent experiments. Symbols are as in Fig. 2.

anisms. Cross-linking of DNA was achieved by photoactivation (UV-A irradiation) of 8-MOP intercalated into DNA. The amount of cross-links formed was controlled by the UV-A dose. As illustrated in Fig. 4 the presence of the human UbcH1 substantially increased the resistance of cells to photoactivated 8-MOP. Compared to rad6 mutants, survival was enhanced about 500-fold at a UV-A dose of 1.5 kJ/m^2. This suggests an important function of UbcH1 in the repair of cross-linked DNA.

In all these complementation experiments, expression of the human UbcH1 in rad6 mutants never restored DNA repair processes to wild-type levels. This should not be due to the different expression levels of wild-type RAD6 and UbcH1 as it is known that expression levels of RAD6 can vary over a large range without affecting normal DNA repair processes. Neither over-expression of RAD6 from the strong GAL1 promoter [30] nor 20-fold lower expression than in wild-type cells [31] influences RAD6 function with respect to DNA repair. Thus, the evolutionary distance between yeast and human, and the susceptibility of protein–protein interactions to amino acid substitutions, seems to hamper complete functional complementation.

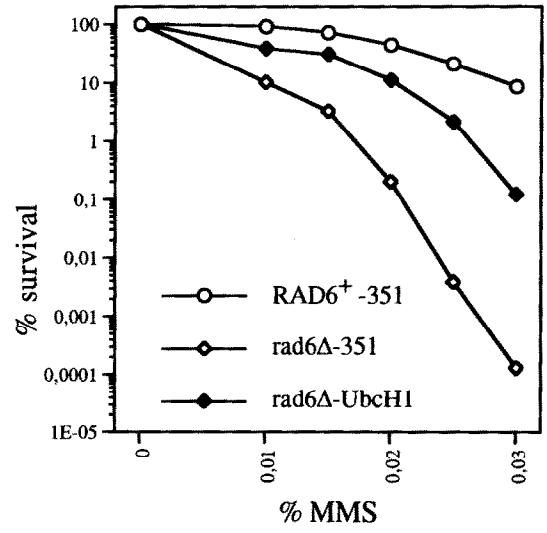

Fig. 3. Survival of yeast transformants treated with the indicated concentrations of methylmethanesulfonate. Results represent 3 separate experiments. Symbols are as in Fig. 2.

However, the experiments described above reveal a substantial role of the human ubiquitin-conjugating enzyme in the recovery of cells from various DNA damage. Although the functions of the human UbcH1 in DNA repair processes had been demonstrated in *S. cerevisiae*, we expected similar functions in mammalian cells. To verify this, UbcH1 deletion mutants would be of special interest, however, the existence of at least two highly homologous *UbcH1* genes [15] might hamper gene disruption experiments. Nevertheless, an important role of UbcH1 in the repair of UV-damaged, alkylated and cross-linked DNA, as well as functions in mutagenesis [15], seems very likely. The question of the molecular basis of these UbcH1 functions arises. One can speculate that UbcH1 mediates the degradation of transcriptional repressors, which regulates a set of DNA repair genes, by the ubiquitin-dependant proteolytic pathway: a function that would be analogous to that of the proteolytically active recA in *E. coli*, which induces SOS repair by degradation of the lexA repressor protein [29]. Alternatively, ubiquitination of histones, which is carried out by UbcH1 in vivo, may modulate chromatin structure and facilitate DNA repair processes. Experiments to investi-

gate these possible mechanisms, namely construction of a mutant UbcH1 lacking specific histone ubiquitination activity and analysis of their ability to substitute the yeast RAD6/UBC2 functions in DNA repair, are currently in progress.

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